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Attempt to Obtain Greater Dermal Depth of Vascular Injury Using Dye-Enhanced Laser Technique: A New Approach

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Background and Objective: **Although pulsed dye laser has been successfully used in the treatment of portwine stains, a number of patients had incomplete clearance because the depth of penetration by the pulsed dye laser was inadequate. This study was performed to establish the greater penetration depth of vascular injury using a dye-enhanced laser technique.**
Study Design/Materials and Methods: **The ruby laser at 694.3 nm was used, and the corresponding specific dye was Prussian blue solution (maximum absorbance 694 nm). We compared the penetration depth of vascular injury by the ruby laser irradiation after the Prussian blue injection with that by the dye laser irradiation. A flashlamp dye laser with a pulse duration of 300 μsec and a 5 mm diameter spot size was used to 6.2 J/cm^2 at 590 nm. The Prussian blue solution was injected into the superficial epigastric artery of white male Japanese rabbits, immediately followed by the ruby laser exposure to 6.2 J/cm^2 at a pulse duration of 283 μsec in a 15 × 15mm spotsize. Depth of penetration was measured from the dermoepidermal junction to the deepest site of vascular injury at 24 hours after laser exposure.**
Results: **Mean penetration of 590 nm of the dye laser light was 1.45 mm; mean penetration of the 694.3 nm ruby laser irradiation after the Prussian blue injection was 2.33 mm. Ruby laser penetration was greater than that of the dye laser.**
Conclusion: **This study emphasizes that the ruby laser irradiation after the Prussian blue injection can induce deeper vascular injury than the dye laser inducing similar pathological changes.**

Key words: dye, photothrombosis, portwine stain, ruby laser

INTRODUCTION

Portwine stains (PWS) have been treated using the pulsed dye laser at 577 nm [1–4]. Recently, the 585 nm wavelength pulsed dye laser has been shown to be superior to 577 nm for the treatment of PWS [5,6]. Although the pulsed dye laser has been successfully used for the treatment of PWS, a number of patients have suffered incomplete clearance of the lesions due to inadequate penetration of the pulsed dye laser. The vascular injury induced by 585 nm irradiation extended to an average depth of only 1.16 mm from the dermoepidermal junction in human PWS skin [5].

This study is aimed at investigating the methods of obtaining greater penetration depth of vascular injury using the dye-enhanced laser technique. The ruby laser at 694.3 nm can penetrate more deeply than the dye laser at 585 nm wavelength [7]; however, the ruby laser is not absorbed efficiently in the vessels. Therefore, we hypothesized that the laser irradiation of vessels af-

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ter the injection of wavelength-specific dyes to enhance absorption could facilitate deeper vascular injury. This report describes the model of application of a new technique to obtain the deeper vascular injury with the use of Prussian blue dye solution (maximum absorbance 694 nm) injected into the rabbit abdominal vessel.

MATERIALS AND METHODS

White male Japanese rabbits, weight 3.0–4.0 kg, were used. The animals were anesthetized with pentobarbital sodium (25 mg/kg). The abdominal skin was depilated using a depilation cream. The dye preparation consisted of the mixture of 0.1 g of Prussian blue (Aldrich Chemical Co., Milwaukee, WI) and 2 ml 0.9% sodium chloride irrigation solution. Prussian blue dye was chosen because of its absorption wavelength of 694 nm, which corresponds to that of the ruby laser. Relative absorption of Prussian blue was determined during this experiment (at 694 nm according to the published data). The absorption of hemoglobin was taken from the literature with slight modification [7].

The animals were divided into four groups. We used five rabbits for each experimental group. The four studies were performed on separate rabbits. Group 1: conventional irradiation with the dye laser to the abdominal skin. The flashlamp dye laser Model DO101 at pulse duration of 300 μsec was applied to spot a 5 mm in diameter at 6.2 J/cm^2 at 590 nm. Group 2: injection of the Prussian blue solution only into the abdominal vessel. A 27-gauge catheter was placed into the superficial epigastric artery, and the Prussian blue solution was injected (2 ml). Group 3: irradiation of the abdominal skin by a ruby laser. A 15 $\times$ 15 mm area of the abdominal skin was exposed to 6.2 J/cm^2 at 694.3 nm using the Toshiba ruby laser (LRT-301A) at a pulse duration of 283 μsec. Group 4: ruby laser irradiation of the vessels after Prussian blue was injected to enhance absorption. A 27-gauge catheter was placed into superficial epigastric artery. The Prussian blue solution was injected and immediately followed by ruby laser irradiation to the abdominal skin. We chose fluences of 6.2 J/cm^2 because the Ruby laser we used had been fixed at 6.2J/cm^2, which corresponded to that for the best clinical effects.

Skin biopsies were taken in each group, immediately after and 24 hours after laser exposure. The tissue was fixed in 10% formaldehyde and processed for light microscopic examination and stained with eosin and hematoxylin on paraffin section. Penetration depth was measured from the dermoepidermal junction (the epidermal base was estimated in a straight line) to the deepest site of vascular injury.

RESULTS

Histologic Observations

Group 1 (dye laser irradiation). Immediately after the laser exposure, the histologic changes included obliteration of many vessels by aggregated red blood cells, endothelial cell injury, vascular rupture, hemorrhage, and denaturation of the perivascular collagen fiber. Twenty-four hours after the laser exposure, infiltration of neutrophils was noted around the vessel necrosis (Fig. 1).

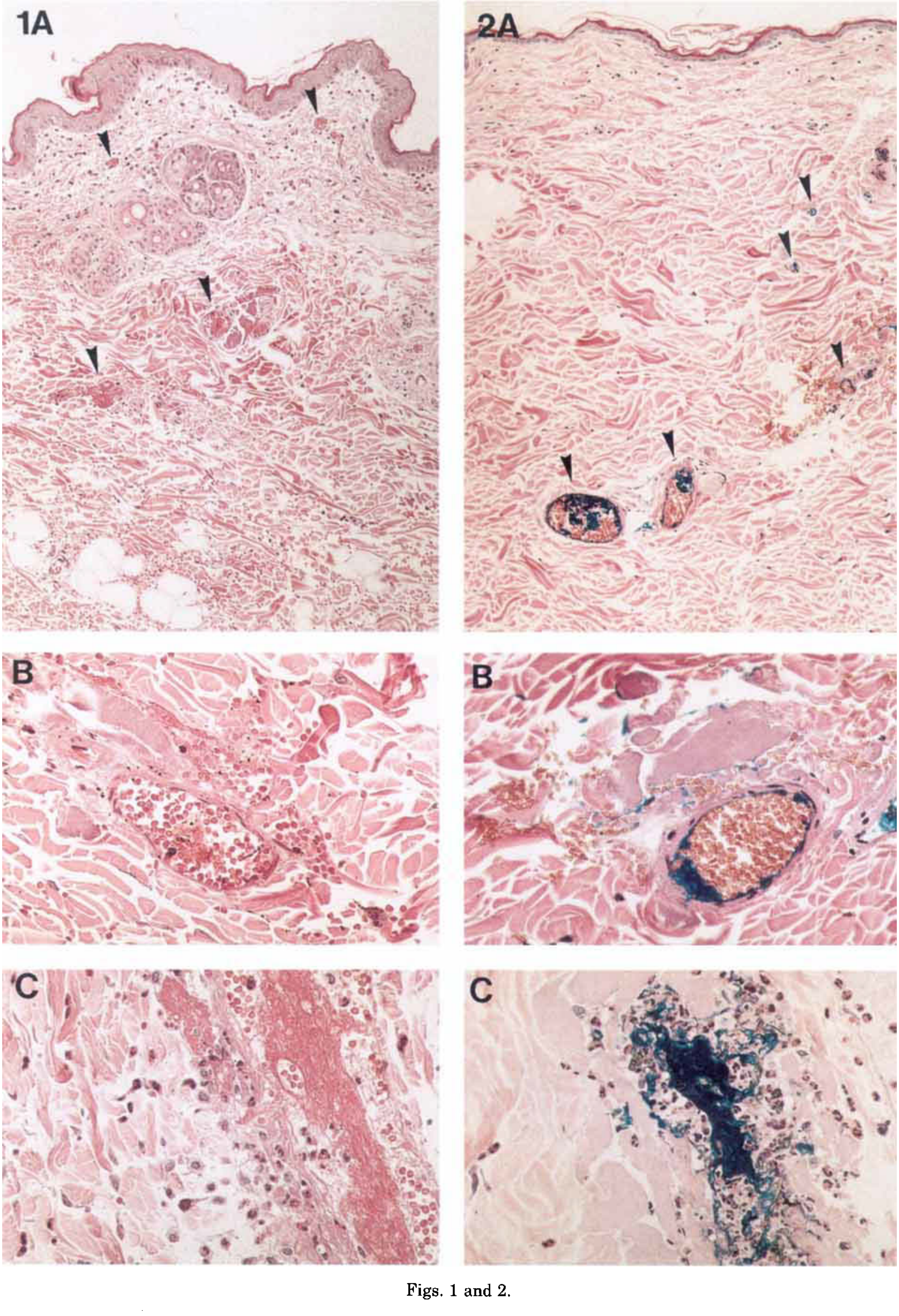
Group 2 (Prussian blue injection only). Immediately after the injection, the Prussian blue dye was noted in the lumen of almost all vessels. After 24 hours, in a considerable number of vessels the Prussian blue dye disappeared, but its focal remnants were noted.

Group 3 (ruby laser irradiation). Immediately after and 24 hours after the laser exposure, there were no remarkable changes in the blood vessels.

Group 4 (ruby laser irradiation after Prussian blue injection). Immediately after the laser exposure, the changes similar to those in Group 1 were found, i.e., histologic changes included obliteration of many vessels due to aggregated red blood cells, endothelial cell injury, vascular rupture, hemorrhage, and denaturation of perivascular collagen fiber. There was Prussian blue dye in the obliterated vessels. Tissue injuries in group 4 were wider in area and deeper than those in group 1. Twenty-four hours after laser exposure, infiltration of neutrophils was seen around necrotic vessels (Fig. 2).

Penetration Depth

Penetration depth was measured from the dermoepidermal junction to the deepest site of vascular injury 24 hours after laser exposure. The criteria of vascular injury included obliteration of vessels by agglutinated red blood cells, endothelial cell injury, vascular rupture, hemorrhage, and denaturation of perivascular collagen fiber as mentioned above. Ten specimens were examined in each group (data presented in Table 1). Mean



Figs. 1 and 2.

TABLE 1. Penetration Depth of Vasucular Injury, Dye Laser Irradiation, and Ruby Laser Irradiation After Prussian Blue Injection

	Dye laser	Prussian blue + ruby laser
No. 1	0.95 mm	1.70 mm
2	1.00	1.75
3	1.05	1.80
4	1.10	1.90
5	1.15	2.15
6	1.65	2.25
7	1.85	2.30
8	1.90	2.35
9	1.90	2.70
10	1.95	2.75
Average	1.45 mm	2.33 mm

penetration depth of the 590 nm dye laser light was 1.45 mm; mean penetration depth of the 694.3 nm ruby laser irradiation after the Prussian blue injection was 2.33 mm. It should be emphasized that ruby laser penetration depth was greater than the depth of dye laser penetration.

DISCUSSION

This study revealed that the ruby laser irradiation after the Prussian blue injection induced vascular injuries similar to those induced by dye laser irradiation, although the remnants of Prussian blue were found in the obliterated vessels. In addition, these vascular injuries were significantly deeper than those after the dye laser irradiation. And these vascular injuries covered wider areas than those of induced by the dye laser irradiation, because the ruby laser has the wider spot size than that of dye laser spot size.

In this study, the conditions of the ruby laser irradiation were similar to those of the dye laser. However, our results demonstrated that the laser with longer wavelength induced the greater depth of vascular injury when combined with injection of the Prussian blue dye. Although the 590 nm laser light did not penetrate as deeply as that of 585 nm [6], the difference of the penetration depth in our study was considered negligible at this energy level. We chose 590 nm simply for technical reasons. Although there was a reference demonstrating the difference of depth in vascular injury between 585 nm and 590 nm, the depth of vascular penetration by 694 nm was anticipated to be much greater than those obtained by either 585 nm or 590 nm [7].

There have been previous studies of dye-enhanced laser techniques using He-Ne laser and Evans blue [8], He-Ne laser and Chicago (Niagara) sky blue [9], and Argon laser and rose bengal [10]. These studies also induced vascular injuries. However, they did not use percutaneous laser irradiation. Libutti et al. [11] investigated ablasion of rabbit ear veins using a dye-enhanced laser technique. To enhance the absorption of the diode laser energy at 808 nm, in their studies the veins in the rabbit's ears were infused with indocyanine green dye (maximum absorption, 805 nm). In the histologic examinations, the vascular wall appeared thinner, the extracellular matrix was charred, and thermally damaged areas surrounded the vessel, although they did not extend to the epidermis. These vascular injuries were similar to those found in our histologic examination of the ruby laser and the Prussian blue.

In this study, we used the Prussian blue because of its maximum absorbance wavelength corresponding to the ruby laser. The Prussian blue is well known as ferric ferrocyanide, $Fe_4[Fe(CN)_6]_3$, used in the paint. However, to our best knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated. In the rabbit, the amount of the Prussian blue used in this study was not fatal. This study is preclinical investigation; a search for new nontoxic chemicals remains to be accomplished.

We consider that the dye-enhanced laser technique would be the most effective treatment for portwine stain. Our study may provide a good

Fig. 1. Histologic features of rabbit skin immediately after dye laser exposure. **A.** Many vessels are disrupted by agglutinated red blood cells and denaturation of perivascular collagen fiber (arrowheads). **B.** Vascular wall and endothelial cells are injured. Vascular rupture, hemorrhage, and denaturation of perivascular collagen fiber are noted. **C.** Twenty-four hours after laser exposure, infiltration of neutrophils is seen around vessel injury.

Fig. 2. Histologic features of rabbit skin, ruby laser irradiation after Prussian blue injection. **A.** Prussian blue dye is seen in the obliterated vessels. Many vessels are disrupted by agglutinated red blood cell and denaturation of perivascular collagen fiber (arrowheads). The depth of the vascular injury is greater than that seen in Figure 1, although histologic findings are similar. **B.** There are Prussian blue dye in the obliterated vessels. Vascular wall and endothelial cells are injured. Perivascular hemorrhage and denaturation of perivascular collagen fiber are seen. **C.** Twenty-four hours after laser exposure, infiltration of neutrophils is seen around the vessel injury.

basic model for determining the most clinically efficient technique.

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